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Development of a novel drug delivery system, time-controlled explosion system (TES): V. Animal pharmacodynamic study and human bioavailability study

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Abstract

FK409, a new vasolidator, was applied to a time-controlled explosion system (TES). After oral administration of the TES with a lag time of 3 h to conscious dogs, both the blood FK409 level and blood pressure were monitored. FK409 appeared in the blood at 3 h and the maximum level was attained at 5 h according to the in vitro release profile. In response to the blood concentration profile of FK409, the blood pressure predominantly decreased after 3 h and minimized around 5 h. A human bioavailability study was also performed, using the TES with a lag time of 3 h. The in vivo lag time for the appearance of drug in the blood was consistent with the in vitro lag time for drug release. Compared with a rapid release formulation, the extent of FK409 absorbed did not decrease and the blood concentration profile was completely separated. Furthermore, the blood concentration profile was not influenced by food intake. These results suggest that the novel drug delivery system, the TES, has potential for use as a preparation of FK409 with prolonged activity.

Key words: Time-controlled explosion system (TES); Lag time; Blood level; Blood pressure; Human bioavailability study; FK409

1. Introduction

FK409 [(3*E*)-4-ethyl-2-hydroxyimino-5-nitro-hexenamide] is a new vasodilator (Hino et al., 1989a), which is isolated from the acid-treated fermentation broth of *Streptomyces griseosporeus* No. 16917 as a semi-artificial fermentation product with vasodilatory and anti-platelet activities (Hino et al., 1989b). This drug has been developed as an anti-angina pectoris and anti-hypertension agent via thrice-daily dosing with a rapid release formulation. In a pharmacokinetic study

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using rats, the systemic half-life of FK409 was determined as 1.6 h, and the absolute oral bioavailability was found to be 20.8% due to first-pass metabolism, since complete absorption was observed in an absorption study using [^{14}C]FK409.

In those cases where the drug is exposed to first-pass metabolism, slow dissolution would result in extremely low systemic drug levels as compared to rapid dissolution. Therefore, slow dissolution requires a much greater dose in order to maintain drug levels within the therapeutic range. In contrast to slow dissolution, pulsatile rapid release could overcome such problems.

We have recently been developing a time-controlled explosion system (TES) which consists of a core, drug, swelling agent and water-insoluble membrane, from the center outwards (Ueda et al., 1994a). In vitro drug release from the TES was initiated by destruction of the outer membrane and the period of time needed for disruption of the membrane is created as a lag time in the drug release profile (Ueda et al., 1994a–c). Furthermore, the lag time can be freely controlled by changing the thickness of the outer membrane (Ueda et al., 1994c). As previously reported (Ueda et al., 1994d), the in vitro drug release of the TES, i.e., membrane destruction and subsequent drug release, is reproduced in in vivo conditions.

The aim of this study was to investigate the feasibility of the TES for a long-acting preparation of FK409 as twice-daily treatment. After oral administration of the TES particles loaded with FK409 to conscious dogs, the blood FK409 levels were monitored with simultaneous measurement of the blood pressure. In order to evaluate the reproducibility of the in vitro drug release behavior on the absorption profiles, a human bioavailability study was carried out using the TES.

2. Materials and methods

2.1. Materials

FK409 (Fujisawa Pharm., Japan) was used as the drug. Sucrose seeds (Nonpareil® 24–32mesh, Freund Industrial, Japan) were employed as cores. Low-substituted hydroxypropylcellulose (L-HPC® LH31 grade, Sin-etsu Chemical Co., Japan) and ethylcellulose 10 cps (Ethocel®, Dow Chemical Co., Japan) were used as the swelling agent and membrane, respectively. All other chemicals were of analytical grade and used without further purification.

2.2. Preparation of the TES

Sucrose seeds were stepwise coated with pulverized FK409, sieved powder of L-HPC and ethylcellulose as described previously (Ueda et al., 1994a).

2.3. In vitro drug release study

An in vitro drug release study was performed according to the JP XII paddle method. Distilled water (900 ml) prewarmed to 37 ± 0.2°C was used as the test medium. The amount of FK409 released was assayed by measuring the UV absorbance at a wavelength of 254 nm.

2.4. Animal pharmacokinetic and pharmacodynamic study

Beagle dogs of either sex, weighing 11 and 12 kg, with an artificial loop in the carotid artery were used under unanesthetized and unrestricted conditions. After oral administration of a rapid release formulation or the TES to dogs at a dose of 10 mg FK409/kg, blood samples were taken at predetermined intervals. The blood samples were stored at −20°C until analysis. To measure blood pressure, a fine Teflon tube filled with heparin solution was inserted into the artery at the loop, and was connected to a pressure-transducer system (telemetry receiver: Model ZR601G, Nihon Koden Ltd; amplifier: Model AP-630G, Nihon Koden Ltd; recorder: Model 8K21, NEC San-ei Ltd).

2.5. Analytical procedure

The blood concentration of FK409 was determined by GC/mass spectrometry using [^{15}N]-

FK409 as an internal standard as previously reported (Ishibashi et al., 1991). The GC/MS consisted of a gas chromatograph (model 9610, Finnigan-MAT Co. Ltd) with helium flow as a carrier gas at 10 lb/inch^2, a capillary column (Ultra 2, 12.5 m × 0.31 mm i.d., coating thickness 0.52 μm, Hewlett Packard Co. Ltd.) and a mass spectrometer (model 4510, Finnigan-MAT Co. Ltd). The detection limit was 0.1 ng/ml using a 2 ml aliquot of blood.

2.6. Human bioavailability study

Subjects

Male healthy volunteers in a group of nine (mean age (±S.E.) 21.8 ± 2.4 years, range 20–28 years; mean weight 59.2 ± 6.1 kg, range 54–70 kg) participated in this study. The subjects were prohibited from smoking during each study, and abstained from alcohol and medication during and for 3 days before each study. Written informed consent was obtained following approval by the Institutional Review Boards of the Osaka Pharmacology Research Clinic in June 1991.

Procedure

This study was performed according to a three way cross-over design. Volunteers fasted overnight (for at least 12 h) and received the TES containing 40 mg FK409 together with 200 ml of water. On other occasions, at 09:00 after fasting, volunteers were given a light breakfast (1930 kJ) consisting of one egg, two pieces of crisp bread, 5 g margarine, 20 g cheese, 200 ml orange juice and 150 ml low-fat milk according to a standardized breakfast (Melander, 1978). Immediately after breakfast, each volunteer received a rapid release formulation or the TES containing 40 mg FK409 together with 200 ml of water. The order of treatments was randomized, each treatment being separated by a period of 7 days. Blood specimens were obtained before and at the following times after oral administration: 0.25, 0.5, 1, 1.5, 2, 3, 4, 5 and 24 h for the rapid release formulation, and 2, 3, 4, 5, 6, 7, 8, 10, 12, 14 and 24 h for the TES.

Statistical analyses

Analyses of variance (ANOVAs) were performed for the individual peak concentration (C_{max}), time of C_{max} (T_{max}), area under the blood concentration curve (AUC), mean residence time (MRT) and lag time, Tukey's multiple range test also being performed.

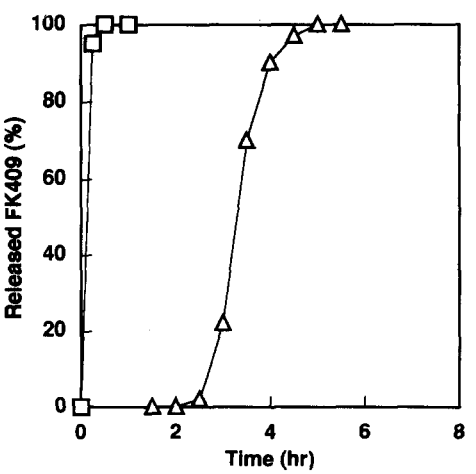

Fig. 1. In vitro release profiles of FK409 from a rapid release formulation (□) and the TES (△) in water.

3. Results

3.1. Animal pharmacokinetic and pharmacodynamic study

Fig. 1 shows the in vitro FK409 release profiles of a rapid release formulation and the TES. For the rapid release formulation, FK409 was found to be immediately dissolved. In the case of the TES, the release of FK409 was initiated at 3 h and completed within 5 h.

The preparations, i.e., the rapid release formulation and the TES with a lag time of 3 h, were administered orally to a male and a female conscious dog at a dose of 10 mg FK409/kg. The blood pressure was continuously monitored, while the blood was collected at predetermined intervals. Fig. 2 and 3 exhibit the time courses of blood FK409 levels and blood pressure, respec-

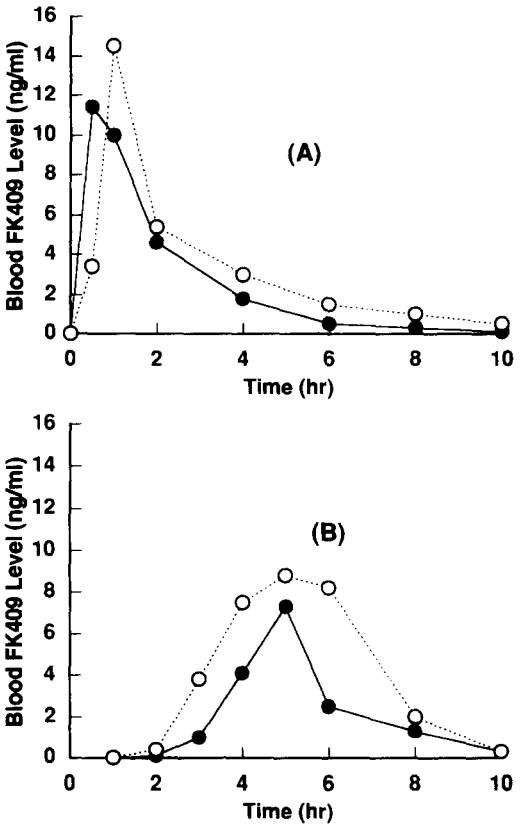

Fig. 2. Time courses of blood FK409 levels after oral administration of a rapid release formulation (A) and the TES (B) at a dose of 10 mg FK409/kg to male (●) and female (○) conscious dogs.

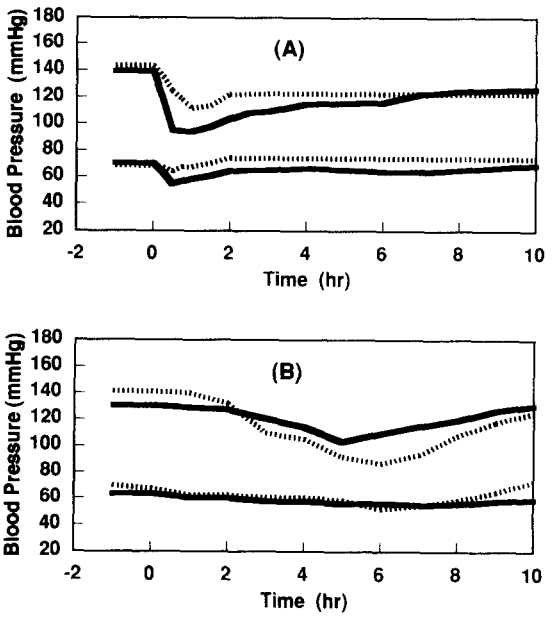

Fig. 3. Time courses of blood pressure after oral administration of a rapid release formulation (A) and the TES (B) to male (solid line) and female (dotted line) conscious dogs. Upper and lower lines represent the systolic and diastolic blood pressure, respectively.

tively. The rapid release formulation showed maximum FK409 blood levels and minimum blood pressure within 1 h without a lag time. In the case of the TES, FK409 appeared in the blood around 3 h and then the maximum drug level was observed at 5 h, corresponding to the in vitro release profile. The blood pressure scarcely decreased within about 2 h, and maximum depression was observed around 5 h followed by a gradual increase. Table 1 summarizes the pharmacokinetic parameters determined in this study. In the case of the TES, almost the same AUC value was observed, however, the C_{max} was decreased due to the slow release rate as compared with the rapid release formulation.

3.2. Human bioavailability study

Table 2 describes the in vitro release profile of FK409 from the TES used in the human bioavailability study. Drug release was complete within 3 h after a lag time of 3 h.

Fig. 4 shows the time courses of mean blood FK409 levels after the oral administration of the TES, as compared with a rapid release formulation. In the case of the TES, the appearance of FK409 in the blood was initiated around 4 h and

Table 1
Pharmacokinetic parameters in dogs at a dose of 10 mg FK409/kg

Preparation	Sex	C_{max} (ng/ml)	T_{max} (h)	$AUC_{0-10\,h}$ (ng h ml^{-1})
Rapid formulation	male	11.3	0.5	25.5
	female	14.5	1.0	31.5
TES	male	7.2	5.0	19.5
	female	8.8	5.0	37.6

Table 2
Release behavior of FK409 from TES used in human bioavailability study [a]

Time (h)	Released FK409 [b] (%)
2	0.0 ± 0.0
3	1.4 ± 0.3
4	32.5 ± 2.6
5	81.0 ± 3.4
6	97.0 ± 2.7
7	100.0 ± 0.0
8	100.0 ± 0.0

[a] Dissolution medium: distilled water.
[b] Each value expressed as the mean ± S.D. of five determinations.

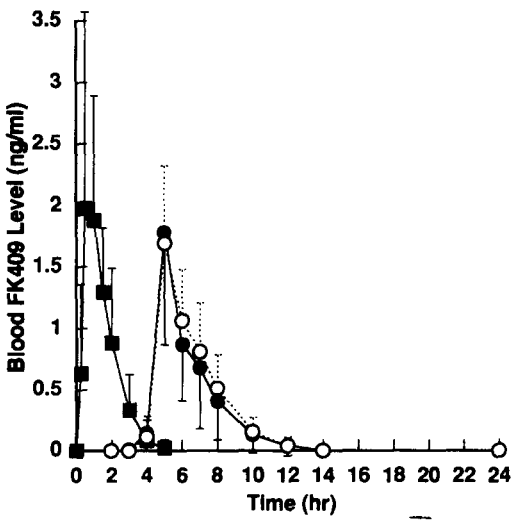

Fig. 4. Time courses of blood FK409 levels after oral administration of a rapid release formulation and the TES to nine healthy volunteers at a dose of 40 mg FK409. Rapid release formulation in the fed state (■); the TES in the fed state (○); the TES in the fasted state (●). Bars denote S.D.

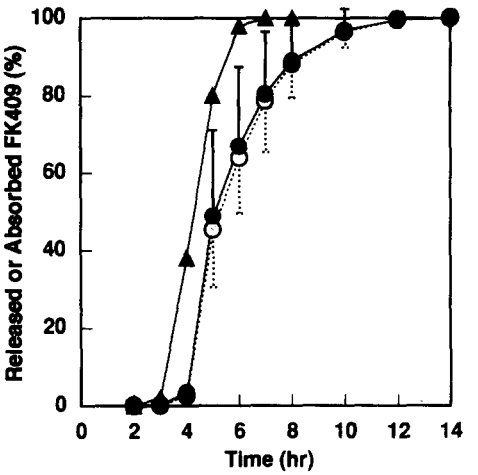

Fig. 5. Comparison between in vitro release and in vivo absorption profiles. In vitro drug release (▲); in vivo drug absorption in the fed state (○); in vivo drug absorption in the fasted state (●). Bars denote S.D.

the blood concentration profiles were found not to be dependent on feeding. Table 3 summarizes the pharmacokinetic parameters obtained from the blood concentration profiles. The pharmacokinetic parameters for the TES such as C_{max}, T_{max}, AUC, MRT and lag time were not influenced by the ingestion of food. Compared with the rapid release formulation, the AUC value was almost identical whereas the MRT significantly increased.

The in vivo absorption profiles of FK409 for the TES were calculated according to the method of Wagner and Nelson (1964) using the systemic elimination rate (1.43 h^{-1}) determined from the mean blood concentration profile of the rapid

Table 3
Pharmacokinetic parameters [a] in human at a dose of 40 mg FK409

Parameter	Rapid formulation	TES		Statistics
	Fed (A)	Fed (B)	Fasting (C)	
C_{max} (ng/ml)	2.62 ± 1.25	1.82 ± 0.86	1.72 ± 0.60	$A = B = C, (1-\beta) = 0.20$
T_{max} (h)	1.00 ± 0.60	5.33 ± 1.00	5.22 ± 0.67	$A < (B = C), (1-\beta) = 0.15$
$AUC_{0-24\,h}$ (ng h ml^{-1})	3.58 ± 1.12	4.43 ± 1.55	4.83 ± 1.52	$A = B, B = C, C > A, (1-\beta) = 0.26$
MRT (h)	1.44 ± 0.37	6.31 ± 0.85	6.41 ± 0.50	$A < (B = C), (1-\beta) = 0.12$
Lag time [b] (h)	0.28 ± 0.08	4.11 ± 0.60	4.44 ± 0.53	$A < (B = C), (1-\beta) = 0.13$

[a] Each value expressed as the mean ± S.D. of nine volunteers.
[b] Lag time defined as the sampling time of the initial detection of FK409 in the blood.

release formulation. The results are depicted in Fig. 5 together with the in vitro release profile. It was ascertained that the absorption profiles ran parallel to those of release.

4. Discussion

The time-controlled explosion system (TES) possesses the property of time-controlled drug release with a pre-designed lag time (Ueda et al., 1993a–d). The aim of this study was to assess the feasibility of using the TES for a long-acting preparation of FK409 as a twice-daily treatment.

In order to investigate the response of the anti-hypertensive effect to the blood FK409 level, both the blood level and blood pressure were monitored after oral administration of the TES with a lag time of 3 h to conscious dogs. Corresponding to the blood levels of FK409, a decrease in blood pressure was observed after 3 h and reached a maximimum at 5 h following the gradual increase (Fig. 2 and 3). It was clarified that the drug release behavior of the TES can be reproduced in the pharmacological response.

The human bioavailability study was performed using the rapid release formulation and the TES with a lag time of 3 h. After oral administration of the TES, FK409 was detected in the blood around 4 h in response to the in vitro lag time (Fig. 4). The AUC and C_{max} were almost equal to those of the rapid release formulation (Table 3). The results indicate that the TES can release the drug in the human alimentary tract in a manner similar to that in vitro. Furthermore, none of the pharmacokinetic parameters for the TES were influenced by the ingestion of food (Table 3). The size of the TES used in this study ranged from 1.0 to 1.5 mm in diameter. Most pellets of such a size are known to be transferred from the stomach to the intestine within 3 h in humans (Davis et al., 1986). It is suggested that the release and subsequent absorption of FK409 in the intestine are not affected by feeding.

Chronobiological studies have demonstrated that temporal patterns exist in the symptomatic intensity and morbidity of human diseases (Smolensky andD'Alonzo, 1988). The TES with a lag time of 3 h provided, with high reproducibility, the maximum blood level around 5 h after administration (Table 3). A night-time dose with the TES would make the blood level attain a maximum early in the morning. Therefore, this system has the potential for use in the chronotherapy of time-dependent diseases, e.g., the severe morning pain of variant angina. In fact, chronotherapy for nocturnal asthma has been conducted by a dose every evening with sustained products containing theophylline in order to maintain a high blood level during the night (Neuenkirchen et al., 1985; Wilkens et al., 1986; Reinberg et al., 1987).

Concerning the TES, a comparison between the profile of in vitro drug release and that of absorption was performed (Fig. 5). The rate of absorption was consistent with that of release in the initial phase, but decreased after 6 h. The observed decrease is likely to be due to a change in the absorption behavior between the small and large intestine, associated with GI transit of the system. A similar change in the intestinal absorption of metoprolol has been observed for an OROS® system (John, 1990).

It has been reported that a constant systemic level of nitrate results in tolerance of the anti-ischemic effect (Abrams, 1980; Leier, 1985; Silber et al., 1987). The blood FK409 profile of the TES with a lag time of 3 h was completely separated from that of a rapid release formulation. Therefore, the combination of a rapid release formulation with the TES should result in a pulsatile blood profile of FK409. Hence, from the therapeutic point of view, such a combined dosage form should possess advantages over the conventional sustained release dosage forms.

5. Conclusion

Twice daily treatment with FK409 using a combination of a rapid release formulation and the TES possessing a lag time of 3 h could provide four pulsatile peaks in the blood drug concentration profile, analogous to a regimen of four daily doses of a rapid release formulation. Therefore, it is considered that the TES holds

promise as a candidate for use in a long-acting preparation of FK409.

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